MISCELLANEOUS NO. 879

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

COMMITTEE:
Dr. Andervont
Dr. Gardner
Dr. Meier

110 EAST 50TH STREET
NEW YORK, N.Y. 10022

Application For Research Grant

SEP 25 1972

Date: *September 18, 1972*

1. Name of Investigator(s): (include Title and Degree(s))
H. David Mosier, Jr., M.D. Professor of Pediatrics

2. Institution &
Address: *Memorial Hospital Medical Center*
2801 Atlantic Avenue
Long Beach, California 90801

3. Short Title of Project:
Effect of Nicotine on the Rat Fetus

4. Proposed Starting Date: *January 1, 1973*

5. Anticipated Duration of this Specific Study: *Three years.*

6. Brief Descripton of Objectives or Specific Aims:

a. *To determine the metabolic effects of high nicotine levels in the fetus and the role of adrenal medullary function in nicotine effects on lipid metabolism in the fetus.*

b. *To determine the effect of exposure to high levels of nicotine throughout gestation on the fetal heart, blood vessels, and other organs, and,*

c. *To determine the effect of exposure to high nicotine levels during fetal life on postnatal growth and development.*

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7. Give a Brief Statement of your Working Hypothesis:
In the adult, nicotine causes a release of epinephrine by the adrenal medulla which in turn promotes fat mobilization. Our tentative hypothesis is that the same reaction occurs in the fetus.

Source: https://www.industrydocuments.ucsf.edu/docs/ntyl0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

See attached sheets

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

See attached sheets

10. Additional Requirements:

None

11. Biographical sketches of all principal and professional personnel (append)

See attached sheets

12. List of publications: (Five most recent as pertinent) (append)

See attached sheets

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3.

13. Budget (1st year)

A. Salaries (Personnel by names)		% time	Amount
Professional			
H.D. Mosier includes fringe benefit		40%	R
Technical			
Regina A. Jansons includes fringe benefit		100%	REDACTED
		Sub-Total	
B. Consumable Supplies (list by categories)		REDACTED	REDACTED
Animals and animal care	1,750		
Chemicals & radiochemicals	1,000		
Glassware	500		
Photography and art work	300		
Misc.	500		
		Sub-Total	
C. Other Expenses (itemize)		4,050	4,050
Costs of publication	600		
Histology at 2.50/slide	300		
Equipment maintenance and service controls	1000		
		Sub-Total	
D. Permanent Equipment (itemize)		1,900	1,900
Misc.	500		
			500
E. Overhead (15% of A+B+C)			3,784
		Total	29,513

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	REDACTED	3,500	1,700	1000	3857	30,567
Year 3		3,500	1,700	1,000	4050	32,052

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature H. David Mosier — REDACTED

Director of Project

H. David Mosier, Jr., M.D. Telephone

Signature

Business Officer of the Institution

Mr. Jack Weiblen — REDACTED Telephone

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Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	*Effect of nicotine on the rat fetus*	*NIH HD 04425-03 Total awarded $51,167 for 3 years*	*15,556*	*to 10/31/72*
	Same title	*Memorial Hospital Foundation*	*approx $8,000*	*11/1/72 - 5/31/72*
Pending	*Effect of nicotine on the rat fetus*	*NIH Renewal grant HD 04425-04-05-06 Approved by National Advisory Council March 1972; in "hold" for funding - no indication funding will be carried out.*	*04 - $38,021 05 - $26,847 06 - $27,239 07 - None (Amounts approved by Council not funded.)*	*11/1/72 - 10/31/75*

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8. Details of Experimental Design and Procedures:

a. Work in Progress

1) Effects of nicotine on fetal adrenal medullary function

We have set out to measure adrenal content of catecholamine content following a suggestion of Professor A. Jost (Paris) in a personal communication that this may provide as good information on acute release of epinephrine as measurement of plasma levels in the fetus. We are progressing with work along this line using measurements of epinephrine and norepinephrine in fetal adrenals, fetal whole body, and maternal adrenals and maternal plasma after a single injection of nicotine intraperitoneally in the pregnant rat.

Details of the methods are as follows:

Virgin female rats of the Long-Evans strain (Simonsen Laboratories, Gilroy, California) are mated with males of the same strain. Day 0 gestation is the day of appearance of the vaginal plug. The females are then transferred for the duration of the experiment to hanging wire mesh cages 7 x 7 x 10 inches in size. Temperature in the animal room is maintained at 21.6 - 26.7°C. Lights are set for 14/10 hour light/dark cycle. Purina Lab Chow and tap water are given ad lib. On day 20 of gestation the pregnant rats are given an injection of nicotine 1 mg/kg ip. The solution injected contains 1 mg nicotine per cc sterile isotonic saline. Controls are injected similarly with sterile isotonic saline or are not injected (0 time controls). In a study now in progress the animals consist of three non-injected rats considered at time 0, three saline controls, and three nicotine injected rats sacrificed at 5, 10, 15, 30, 45, and 60 minutes after injection. At sacrifice the pregnant rat is decapitated. Neck blood is collected in chilled centrifuge tubes containing 0.2 ml heparin sulfate 1:5000 and 5 mg sodium metabisulfite. The tubes are swirled gently and placed in ice. The abdomen and uterus are rapidly opened, fetuses removed, stripped of membranes, and separated from placentas, quickly swirled in a bath of isotonic saline to clean of blood and amniotic fluid, snapped in perforated plastic capsules and dropped into liquid nitrogen. The maternal adrenals are then removed and put into plastic capsules and then liquid nitrogen. The tissues are removed to pre-chilled screw capped jars and held at -60°C for dissections. The maternal plasma is separated in a refrigerated centrifuge and frozen in vials at -60°C pending determinations.

The procedure for determination of epinephrine and norepinephrine follows the method of Anton and Sayre, J. Pharmacol. Exp. Therap. 138:360, 1962. We have recently modified this to include final re-acidification after conversion to the lutine. The determinations of fetal adrenals and maternal adrenals given in the table below were done with this modification. For dissections of fetal adrenals, the fetuses are allowed to thaw at room temperature and the adrenals are removed with aid of a stereo-zoom dissection microscope. All peri-adrenal fat is removed. Three pairs of adrenals from fetuses of the same maternal rat are combined for each determination. Each pair of maternal adrenals are weighed and combined for determination. The results of fetal adrenal determinations are given as µg per fetus. The fetal adrenals were not weighed

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in this study in order not to delay the procedure and risk degradation of the catechols. Weights of fetal adrenals at 20 days gestation in this strain of animals have been determined in our laboratory previously. These average 2.2 mg for males and 1.7 for females. No attempt was made to sex the fetuses in this series as it seemed unlikely that adrenal medullary volume itself would have a corresponding sex difference.

The results to date of this study are given in the figures a and b and in the descriptive paragraphs below.

An examination of the data indicates that the epinephrine content of fetal adrenals 30 and 45 minutes after nicotine injection is significantly lower than that of the controls. In controls there is an upward trend of fetal adrenal content from 0 time value by 45 minutes. This could be the result of handling and maternal excitement caused by the injection. Nicotine apparently eliminates this rise in adrenal concentration.

Norepinephrine concentration in the fetal adrenal is somewhat more variable. No significant difference is seen between nicotine and saline injected animals. Both, however, appear to have an increase in norepinephrine concentration evident from 10 minutes through 45 minutes. Normal values are regained by 60 minutes.

Maternal adrenals after nicotine injection show a marked drop in epinephrine concentration between 5 and 10 minutes. Norepinephrine changes little after nicotine injection by 10 minutes. Saline controls had a marked drop in epinephrine concentration by 5 minutes. Values from 15 minutes through 60 minutes have shown no differences between nicotine and saline injected animals. This work is preliminary and requires confirmation.

Fetal whole body concentrations of epinephrine and norepinephrine at 15, 30, 45, and 60 minutes show no differences between nicotine and saline injected rats. Determinations are yet to be carried out on 5 and 10 minute specimens.

Maternal blood has not yet been assayed.

This work is still in progress. The results thus far suggest an immediate (5 minutes) reaction of maternal medullary discharge of epinephrine content after nicotine injection. Fetal adrenal response is delayed and seems to consist mainly of a lack of the progressive increase in epinephrine content observed in the saline injected groups. Work is planned to repeat earlier time periods with a larger dose of nicotine, e.g. 5 mg/kg, in order to determine whether the fetal adrenal response may be dose related. This increase in dose should more than compensate for the difference in fetal and maternal plasma concentration. Our earlier studies (see Mosier and Jansons, article in press, Teratology, attached) showed that 5 minutes after iv injection of labelled

10035390 94

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nicotine in maternal rat, the radioactivity concentration in fetal plasma was more than half that of maternal plasma and the proportion of nicotine to metabolites was greater in fetal than maternal plasma. We may carry out a comparison study with iv injections, under pentobarbital anesthesia. This should avoid the portal circulation on the first circulatory passage of nicotine and enhance delivery of the dose to the fetus. Possible interference of the barbituate at the level of the fetal adrenal medulla will be considered in interpreting results.

2) Effects of administration of nicotine throughout pregnancy on fetal plasma, lipid phosphorus, and cholesterol.

Nicotine is added to finely ground Purina Lab Chow to give concentrations of 0.05 mg/g or 0.10 mg/g. From day 0 or from days 10-11 in gestation to day 20 the nicotine diet is given in place. Control animals are maintained on finely ground Purina Lab Chow. Tap water is given ad lib. Food intake and body weight at intervals of 2-3 days are recorded. At day 20 animals are anesthetized lightly by ether fumes, the abdomen is quickly opened and fetuses removed from their membranes. They are quickly blotted, decapitated, and bled into heparinized capillary tubes. The plasma is separated quickly by centrifigation in the cold, and stored at -60°C pending analysis.

a. Lipid phosphorus

Determinations are carried out on 40 microliter quantities of plasma by a modification of the method of Zilversmit and Davis, J. Clin. Lab. Med. 35:155, 1950. In a preliminary study carried out during summer of 1972 the following results were obtained:

Nicotine concentration in diet mg/g	N	Lipid Phosphorus (mg/100 ml) Mean ± SEM
0.05 days 10-20	4	5.648 ± 0.1540
0.10 days 0-20	5	6.079 ± 0.2113
0.10 days 10-20	4	5.246 ± 0.1248
controls	4	5.471 ± 0.4548

Plasma of each litter was pooled; duplicate determinations were carried out per pool. Results of the litters were averaged.

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b. Cholesterol

Plasma from the same series used in the lipid phosphorus study described above is in storage awaiting cholesterol determinations by a micromodification of the method of Henry, Clinical Chemistry, 1964. pp. 856-858.

b. Work Planned

1) Acute effect of nicotine on fetal plasma non-esterified fatty acids (NEFA) and triglycerides.

Pregnant rats on day 20 of gestation will be injected with 1 mg/kg nicotine and sacrificed with fetal bleeding as described above at 10, 20, and 30 minutes after injection. Non-injected (0 time) and saline injected controls will be included. This series is being collected at present. The fetal plasma NEFA will be determined by an adaptation of the method of Elphick, J. Clin. Path. 21:567, 1968. Triglycerides will be determined by an adaptation of the method of Soloni, Clin. Chem. 17:529, 1971.

2) Effect of adrenergic blocking agents on response of fetal NEFA and triglycerides to nicotine.

Groups of pregnant rats at 20 days gestation will be injected intraperitoneally according to one of the following schedules: (a) isotonic saline, two injections one hour apart, or (b) isotonic saline followed in one hour by nicotine 0.1 mg/kg; or (c) an adrenergic blocking agent (see table below) followed in one hour by nicotine 0.1 mg/kg.

TABLE

Blocking Agent	Dose	Receptor Blocked
Phenoxybenzamine	8 mg/kg	Alpha
Pronethalol	5 mg/kg	Beta
Chlorpromazine	5 mg/kg	Central adrenergic reflex

10035390 96

The doses of nicotine and blocking agents will be changed if preliminary measurement of the effect and data of a), above, indicate this would be desirable. At 10, 20, and 30 minutes (with separate groups for each time interval) the abdomen will be opened and the fetuses quickly removed and exsanguinated by methods described above. Determinations will be carried out in duplicate in litter pools of plasma.

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c. Effect of fetal nicotine exposure on cardiovascular renal systems after birth.

Litters of rats exposed to different concentrations of nicotine and controls will be killed at different ages from birth through 121 days of age to determine whether gross or microscopic alterations can be seen in heart, vessels, or kidneys. Weights of organs including heart, kidneys, gonads, and adrenal glands will be measured at these autopsies for comparison with controls.

d. Effect of fetal nicotine exposure on subsequent growth

Litters of pregnant nicotine fed rats and control rats will be observed for 121 days after birth. Nicotine will be stopped at day 20 of gestation. Litters will be cut to 8 pups per mother. Body weight and tail length will be measured at weekly intervals beginning at two days of age. At 121 days, at sacrifice, tibial length and tibial epiphyseal width will be measured.

Figures a. and b.

a. epinephrine and norepinephrine content of fetal adrenals
b. epinephrine and norepinephrine content of fetal whole body

9. Physical facilities available

Office and laboratory - located in the Earl and Loraine Miller Children's Hospital in the Memorial Hospital Medical Center of Long Beach. These include:

Investigator's office
Secretary's office
Biochemical laboratory, bench space, usual outlets, sinks, fume hood.
Room for heavy equipment
Room for sensitive instruments
Storage and equipment room
Room for trainees, calculator, photography, and files
Animal facility with room for animals with controlled air circulation, temperature, lighting, surgical room laboratory, and instrument room; storage room.

Access to certain special facilities in the four-story clinical laboratory wing of Memorial Hospital under the direction of Elmer R. Jennings, M.D. These include also glass washing service provided by the staff of the laboratory.

Animal caretaking is provided by the Department of Medical Education of Memorial Hospital in exchange for a set day rate per animal.

10035390 97

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R: REDACTED MATERIAL

Access to a Control Data 3300 computer facility for specifically approved research requests.

Special equipment now in the investigator's laboratory:

Cahn electrobalance, Mettler semi-micro balance, Roller-Smith torsion balance, Packard Tri Carb Model 3320 liquid scintillation counter and auto-gamma accessory, Packard Model 7201 Radio-chromatogram scanner, IEC Model PR-2 refrigerated centrifuge, Revco ultra deep freezer, Aminco-Bowman spectro-photofluorometer with X-Y recorder, Beckman Model DU-2 direct reading spectrophotometer with Digital Concentration Converter and Linear-Log Recorder; Polaroid MP-3 copier; Hewlett-Packard Model 9100 B program table calculator with X-Y Recorder and Printer.

11. *Biographical sketch of investigator:*

Personal: REDACTED

Education:	*B.S. University of Notre Dame, Notre Dame Indiana*	R
	M.D. The Johns Hopkins University School of Medicine, Baltimore, Maryland	R
Internship:	*Pediatrics, The Johns Hopkins Hospital*	*1952-3*
Residency:	*Assistant Resident in Pediatrics, Los Angeles Children's Hospital*	*1953-4*
	Resident in Pediatric Pathology, Los Angeles Children's Hospital	*1954-5*
Fellowship:	*Fellow in Pediatric Endocrinology, The Johns Hopkins Hospital, Baltimore, Maryland*	*1955-7*
Certification:	*Certified by the American Board of Pediatrics*	*1957*
Licensure:	*Maryland Board of Examiners*	*1952*
	California Board of Medical Examiners	*1958*
Career:	*Assistant in Pathology, University of Southern California*	*1954-5*
	Assistant Professor of Pediatrics, University of California, Los Angeles	*1957-61*
	Associate Professor of Pediatrics, University of California, Los Angeles	*1961-3*
	Consultant in Endocrinology, Pacific State Hospital, Pomona, California	*1957-63*
	Director of Research, Illinois State Pediatric Institute, Chicago, Illinois	*1963-7*
	Associate Professor of Pediatrics, University of Illinois College of Medicine, Chicago, Illinois	*1963-7*
	Senior Consultant, Los Angeles County General Hospital	*1967-8*

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Physician in Charge, Pediatric Endocrine Clinic, Cedars-Sinai Medical Center, Los Angeles 1967-8
Professor of Pediatrics, University of California, Irvine 1967-

12. Selected publications:

Richter, C.P. and Mosier, H.D., Jr.: Maximum sodium chloride intake and thirst in domesticated and wild Norway rats. Am. J. Physiol. 17:213, 1954.

Mosier, H.D., Jr.: The development of the hypothalamoneurohypophyseal secretory system in the chick embryo. Endocrinology 57:661, 1955.

Mosier, H.D., Jr.: Comparative histological study of the adrenal cortex of wild and domesticated Norway rats. Endocrinology 60:460, 1957.

Mosier, H.D., Jr. and Blizzard, E.M.: Defects in the biosynthesis of thyroid hormone in congenital goitrous cretinism. A.M.A.J. Dis. Child. 94:390, 1957. (Abstract).

Blizzard, R.M. and Mosier, H.D., Jr.: Protein binding properties of mono- and diiodotyrosine as compared with thyroxin and triiodothyronine. A.M.A.J. Dis. Child. 94:534, 1957. (Abstract)

Mosier, H.D., Jr., Blizzard, R.M., and Wilkins, L.: Congenital defects in the biosynthesis of thyroid hormone. Pediatrics 21:248, 1958.

Mosier, H.D., Jr., and Richter, C.P.: Response of the glomerulosa layer of the adrenal gland of wild and domesticated Norway rats to low and high salt diets. Endocrinology 62:268, 1958.

Mosier, H.D., and Armstrong, M.K.: Absence of binding of inorganic iodide I^{131} by irradiated human serum protein in vitro. Endocrinology 11:671, 1962.

Mosier, H.D., Jr., Armstrong, M.K., and Schultz, M.A.: Measurement of the early uptake of radioactive iodine I^{131} by the thyroid gland: A method requiring reduced irradiation. Pediatrics 31:426, 1963.

Mosier, H.D., Jr., and Armstrong, M.K.: Effects of maternal intake of nicotine on fetal and newborn rats. Proc. Soc. Exp. Biol. & Med. 116:956, 1964.

Mosier, H.D., Jr.: Presence of the long-acting thyroid stimulator in serum in mongolism without hyperthyroidism. J. Clin. Endocr. 25:1005, 1965.

Mosier, H.D., Jr., Grossman, H. J., and Dingman, H.F.: Physical growth in mental defectives. Pediatrics, suppl., 36:465, 1965.

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Mosier, H.D., Jr.: In vitro incubation of rat and rabbit thyroid with L-tyrosine-C^{14} sodium iodide-I^{131} and L-mono-and diiodotyrosine-C^{14}-I^{131}. Proc. Soc. Exp. Biol. & Med. 121:573, 1966.

Dingman, H.F., Mosier, H.D., Jr., and Grossman, H.J.: Deviation in somatic growth: a factor analysis. Child Development 27:949, 1966.

Mosier, H.D., Jr., and Armstrong, M.K.: Effect of maternal nicotine intake on fetal weight and length in rats. Proc. Soc. Exp. Biol. & Med. 124:1135, 1967.

Mosier, H.D., Jr. and Jansons, R.A.: Stunted growth in rats following X-irradiation of the head. Growth 31:139, 1967

Mosier, H.D., Jr., and Richter, C.P.: Histologic and physiologic comparisons of the thyroid gland of the wild and domesticated Norway rat. Anat. Rec. 158:263, 1967.

Mosier, H.D., Jr.: The neck-thigh radioiodine ratio in mongolism. J. Ment. Defic. Res. 2:97, 1967.

Mosier, H.D., Jr., and Jansons, R.A.: Pituitary content of somatotropin, gonadotropin, and thyrotropin in rats with stunted linear growth following head x-irradiation. Proc. Soc. Exp. Biol. & Med. 128:23, 1968.

Mosier, H.D., Jr.: Thyroid function in mongolism: Evidence for a defect in iodide trapping. Proceedings of XII International Congress of Pediatrics, Mexico City, Dec., 1968. (Abstract)

Mosier, H.D., Jr.: Allometry of body weight and tail length in studies of catch-up growth in rats. Growth 33:319, 1969.

Mosier, H.D., Jr.: Effect of X-irradiation on selected areas of the head of the newborn rat on growth. Radiat. Res. 43:92, July 1970.

Mosier, H.D., Jr.: Causes of failure of catch-up growth after certain forms of growth retardation. Pediat. Res. 4:459, 1970.

Mosier, H.D., Jr.: Failure of compensatory (catch-up) growth in the rat. Pediat. Res. 5:59, 1971.

Mosier, H.D., Jr.: Transplacental passage of nicotine in the rat. Clin. Res. 19:222, 1971. (Abstract)

Mosier, H.D., Jr., and Jansons, R.A.: Transplacental passage of nicotine in the rat. Proceedings of the XIII Internationsl Congress of Pediatrics, Vienna, Austria, August 1971. (Abstract).

Mosier, H.D., Jr., and Jansons, R.A..: Allometry of body weight and tail length after head X-irradiation in rats. Growth 35:23, 1971.

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Mosier, H.D., Jr.: Decreased energy efficiency after cortisone induced growth arrest. Growth *36:123, 1972.*

Mosier, H.D., Jr., Smith, F.G., and Schultz, M.A.: Failure of catch-up growth after Cushing's sundrome in childhood. A.M.A.J. Dis. Child. *124:251, 1972.*

Mosier, H.D., Jr., and Jansons, R.A.: Distribution and fate of nicotine in the rat fetus. Teratology *(In press).*

Dearden, L.C., and Mosier, H.D., Jr.: Hypothyroidism induced by propylthiouracil and its effect on the ultrastructure of tibial epiphyseal chondrocytes during long term recovery. Anat. Rec. *(In press).*

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Fig. 3

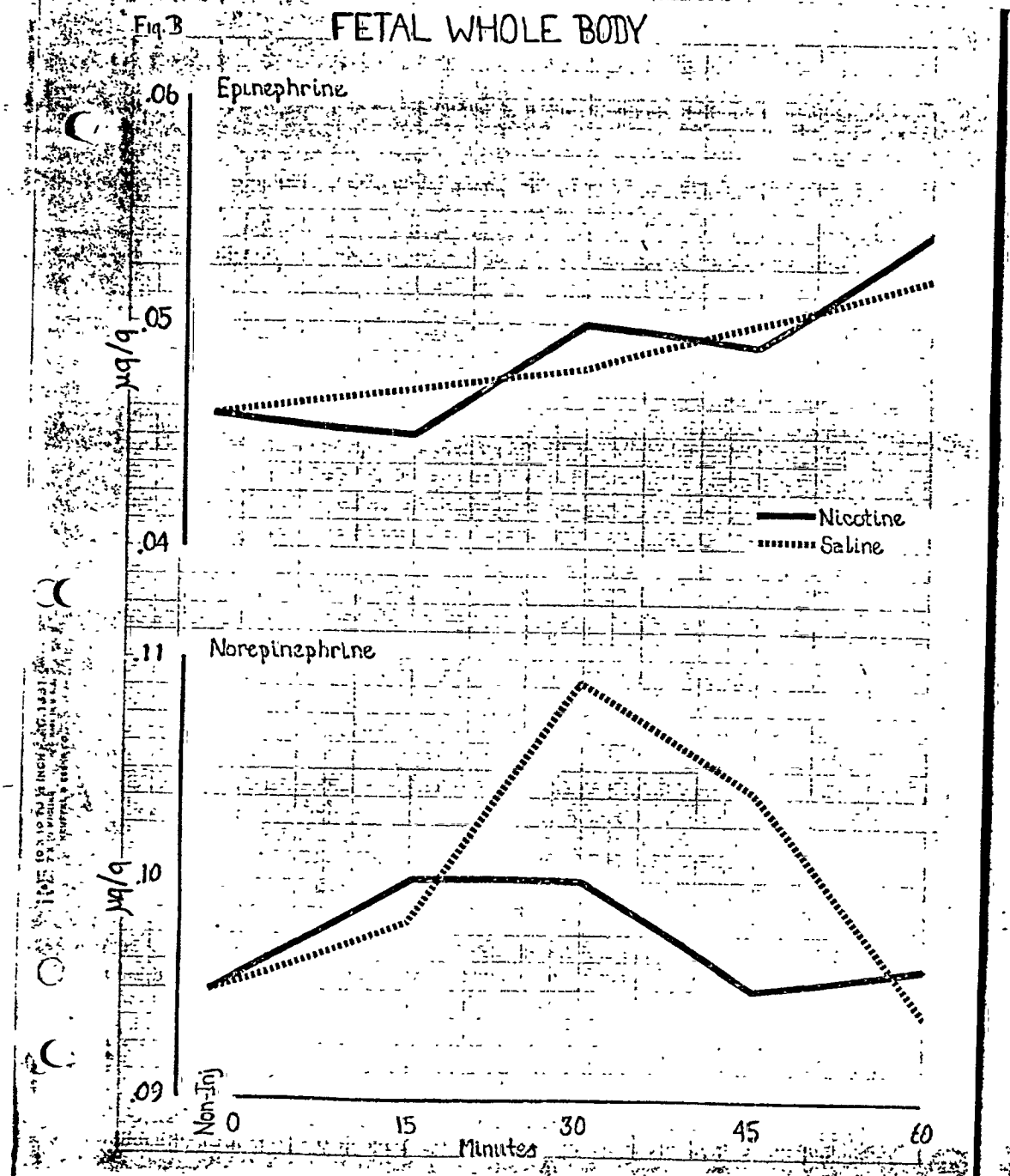

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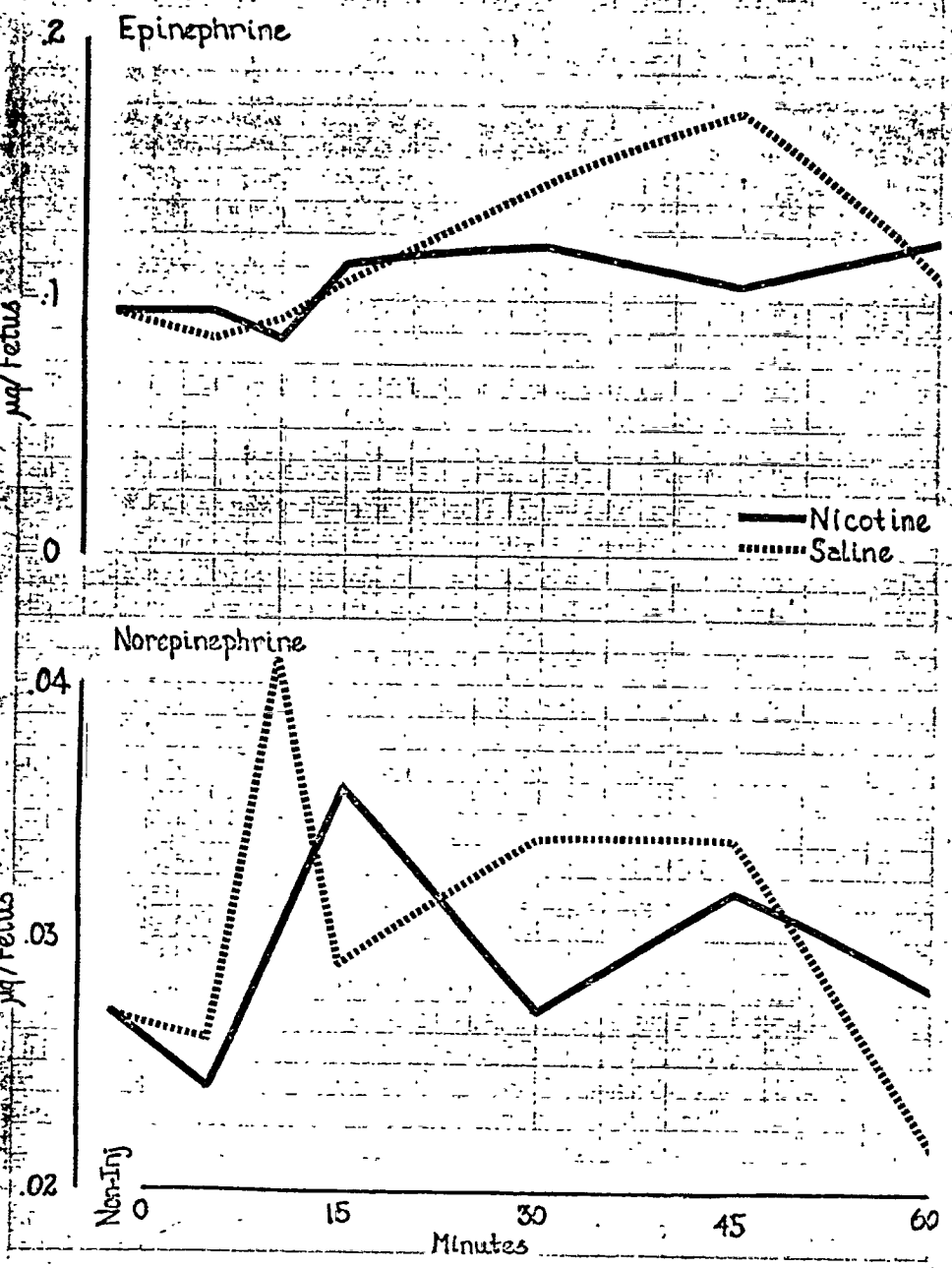
Fig. A. FETAL ADRENALS



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BO-LT. GREY	BA-EXECUTIVE RED
BP-LT. GREEN	BR-BROWN
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BY-YELLOW	BB-ROYAL BLUE

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